



HOLOCENE HISTORY OF ACONITUM IN THE POLISH WESTERN CARPATHIANS AND ADJACENT REGIONS: LONG-DISTANCE MIGRATIONS OR CRYPTIC REFUGIA?

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Abstract. *Aconitum lasiocarpum* (Rchb.) Gayer and *A. variegatum* L. are forest species with overlapped geographical ranges in the Beskid Niski and Doly Jasielsko-Sanockie Depression (W. Carpathians). They form here a hybrid zone. The cytogenetic evidences based on the Giemsa C-banding in *A. variegatum* showed the same cytotype in the Silesian Upland and the Moravskoslezské Beskids, pointing to the role of the Moravian Gate in the migrations of plants from the Moravian glacial forest refugium. Another linked the Małopolska Upland (Ojców) with the two Carpathian regions, including the Pieniny Mts. The result points to the two hypothesis. Firstly, there existed glacial forest cryptic refugia in both regions, or the Małopolska's population is secondary in relation to the Pieniny Mts. ISSR analysis of *A. moldavicum* showed relationships between one of the Małopolska's population and the Podolian populations. The Holocene migrations of the species from the Beskid Niski to the Małopolska region were also probable. The refugial character of the Pieniny Mts. was corroborated by their close relation to the relictual populations of *A. moldavicum* Hacq. from the Nizke Tatry.

Key words: *Aconitum lasiocarpum*, *Aconitum moldavicum*, *Aconitum variegatum*, C-banding, Central Europe, cryptic forest refugia, hybrid zone, PCR-ISSR

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Introduction

The Quaternary glaciations are believed as an important period for genetic diversification and speciation (WILLIS & NIKLAS 2004). At that time the repeated redistribution and isolation of plant and animal species took place, resulting in the origin of cryptic glacial refugia (STEWART & LISTER 2001), hybrid zones (HEWITT 2001), and increased rate of microevolutionary events (KADEREIT *et al.* 2004), linked with the founder effect, genetic drift (BOROŃ *et al.* 2011), and isolation by distance (WRIGHT 1943). The microevolutionary processes in some high-alpine plants did not take place, as originally assumed, in geographical isolation in high-altitude interglacial refugia, but rather at low altitudes in geographically isolated glacial refugia (KADEREIT *et al.* 2004).

Pollen records give evidences on the existence of the European forest “classical” long-term glacial refugia during the last

glacial period in the Near East and the three southern peninsulas of Europe: Balkan, Italian and Iberian (WILLIS & NIKLAS 2004; BENNET & PROVAN 2008). In recent years it is observed a growing body of literature dealt with the responses of terrestrial plants to the climatic oscillations of the Quaternary in the Northern Hemisphere mid-altitude regions. The most interesting biogeographical problem is the existence of so called cryptic refugia encompassed small environmental packets of boreal-temperate trees and herbaceous plants that have survived the last full-glacial period in Central and Eastern Europe. There are now at least 35 localities in Central and Eastern Europe containing evidence from macrofossil wood charcoal assemblages indicative of at least 17 different tree taxa, including some deciduous species as *Alnus* and *Fagus* that could thrive the last pleniglacial in cryptic “northern” refugia (WILLIS & VAN ANDEL 2004; MAGRI *et al.* 2006). They were placed in favorable sites

among a mosaic of geoecosystems, including enclaves of vegetation in the periglacial climate of the full-glacial period (STARKEL 1988).

The other consequence of the expansion and contraction cycles is the origin of contact zones where could meet otherwise isolated species or populations after a postglacial expansion (STEBBINS 1985; TABERLET *et al.* 1998; HEWITT 2001). Such an event can be a clue to the formation of the hybrid taxon. In that way probably originated an Eastern-Sudetic endemic *Aconitum plicatum* Koehler ex Rchb. subsp. *sudeticum* Mitka, that could have joined the genetic stocks of the Carpathian *A. firmum* Rchb. and the Sudetic *A. plicatum* during one of the glacial periods (MITKA *et al.* 2007; MITKA 2012).

The aim of the present paper is to overview, based on the studies carried out on the genus *Aconitum* in recent years (MITKA & KOZIOŁ 2009; ILNICKI *et al.* 2011; SUTKOWSKA *et al.*, in print), some facts and hypothesis dealt with the postglacial history of some species of the genus in the Western Carpathians. The examples encompass problems of the existence of the cryptic refugia (*A. moldavicum* Hacq., *A. variegatum* L.) and the natural hybrid zone (*A. x pawlowskii* Mitka & Starmühl.) in the Western Carpathians and their northern forelands.

***Aconitum moldavicum* Hacq.**

Aconitum moldavicum is a montane species subendemic to the Carpathians, descending to the lowlands (SZAFER 1930). Nowadays, it is circumscribed within the two subspecies: typical subsp. *moldavicum* and subsp. *hosteanum* (Schur) Anderson & Graebn. Recent taxonomical studies revealed that the two subspecies and their spontaneous hybrid *A. m.* subsp. *hosteanum* × *A. m.* subsp. *moldavicum* occur in the lowlands in Poland, including the Małopolska Upland (MITKA & KOZIOŁ 2009). Subsp. *hosteanum* seems to have the center of occurrence on Podolia in the Ukraine, whereas subsp. *moldavicum* in the southern part of the Western Beskidy, i.e. in Slovakia. Such a pattern suggests that the

Małopolska Upland's part of the geographical range of the species originated as an effect of the probable meeting of two migrational elements: the southern (subsp. *moldavicum*) and south-eastern (subsp. *hosteanum*). The time of the presumed meeting is unknown, however taking into consideration a limited dispersal capacity of *A. moldavicum* it could not have happened in the Holocene since the rebuilding of forests in the type of present oak-hornbeam forest *Tilio-Carpinetum* in southern Poland, being the main site of the species, was accomplished c. 4000 years BP (RALSKA-JASIEWICZOWA *et al.* 2004). It seems period too short for re-establishment of subsp. *hosteanum* in the Małopolska Upland from the putative Eastern Carpathian – Podolian refugium (MITKA 2008). The alternative hypothesis is that *A. moldavicum* s.l. have persisted the last glaciation in some of the cryptic refugia in the Western Carpathians and/or in the Małopolska Upland.

To check the hypothesis a pilot investigations on the genetic structure of the species based on PCR-ISSR analysis were carried out. *A. moldavicum* under study originated from the four geographical regions: the Małopolska Upland, Beskid Niski (Western Carpathians), Bieszczady Zachodnie (Eastern Carpathians), Slovakian Western Carpathians, and Podolia (Ukraine). In the effect of classification, the Małopolska Upland joined both with the Beskid Niski Mts., as well as Podolia (Fig. 1). The Slovak populations formed a distinct group, to which was attached the population from the Polish Pieniny Mts. (Mt. Wysoka).

***Aconitum variegatum* L.**

Aconitum variegatum occurs in Western, Central and Southern Europe in the lowlands and in the mountains; for example, in the Tatra Mts. it has been found up to ca. 1700 m a.s.l. Generally, it is a forest species growing on the lowlands in wet broadleaved forest and thickets, along torrents and rivers; in mountains in the montane alderwood *Alnetum incanae* and in tall-herb community *Arunco-Doronicetum* (MITKA 2003). It prefers moderately wet soils with addition of the calcium carbonate. The

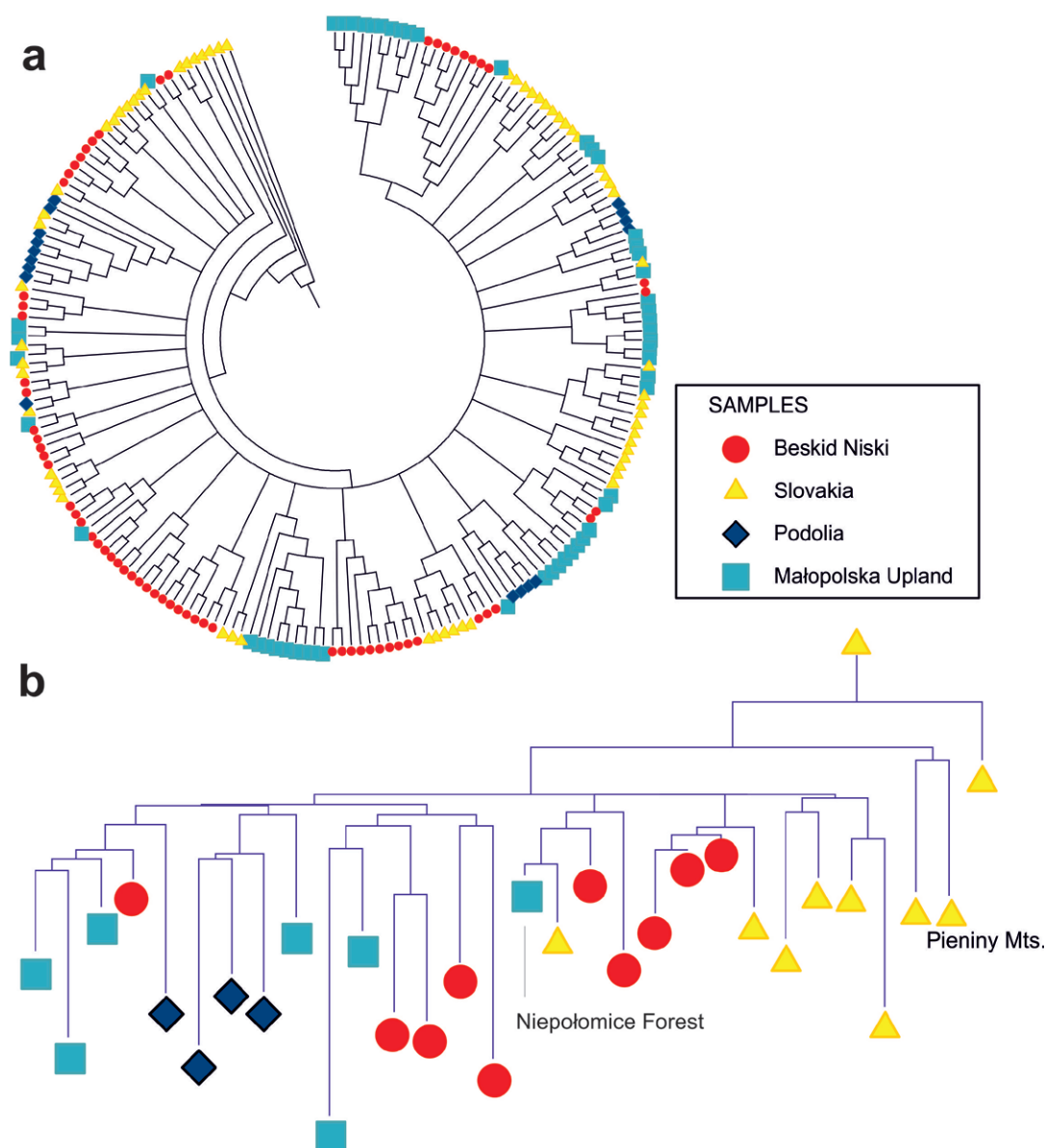


Fig. 1. **a** – Classification UPGMA (PHYLIP, Felsenstein 2006) of 221 individuals *Aconitum moldavium* in W. Carpathians and Podolia based on 473 ISSR bands; **b** – unrooted Neighbour Joining of 31 populations based on the F_{ST} distance (ARLEQUIN ver. 3.0, EXCOFFIER *et al.* 2005).

centre of occurrence of subsp. *variegatum* in Central Europe is in the Western Carpathians, where it has an eastern limit of the geographical distribution in the Beskid Niski (Fig. 3 b).

The UPGMA based on the Reynold's genetic distances revealed the four groups of C-banding pattern, called hereafter Cytotypes

(ILNICKI *et al.* 2011). The Cytotype I, moderately supported with 49.3%, was found in two Western Carpathians populations: in the Moravskoslezské Beskids (Czech Republic) and the Silesian Beskid (Poland) (Fig. 2). The Cytotype II, moderately supported with 56.6%, encompasses the one Polish Upland's

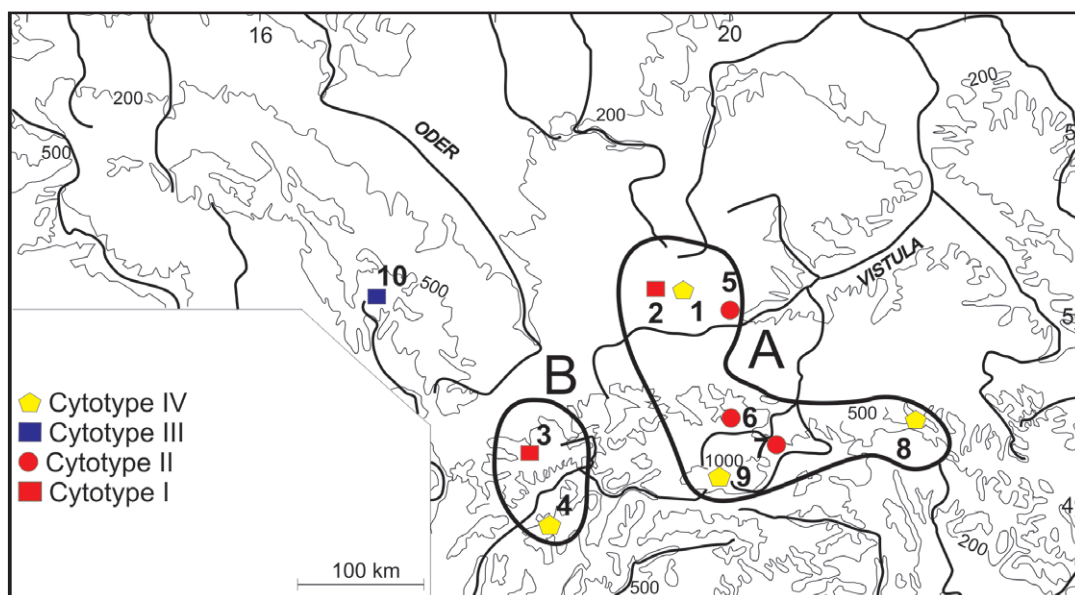


Fig. 2. Distribution of the four C-Giemsa heterochromatine **Cytotypes I-IV** and the two groups (**A** and **B**) based on ISSR analysis and UPGMA classification of *Aconitum variegatum* sampled in the Western Carpathians, Sudetes Mts. and Polish Uplands (ILNICKI *et al.* 2011): **1** – Dąbrowa Górnicza, Silesian Upland; **2** – Bytom-Blachówka, Silesian Upland; **3** – Podolanky, Moravskoslezské Beskid Mts., W. Carpathians; **4** – Súľov, Stražovské vrchy Mts., W. Carpathians; **5** – Ojców, Małopolska Upland; **6** – Rzeki, Gorce Mts., W. Carpathians; **7** – Mt. Wysoka, Pieniny Mts., W. Carpathians; **8** – Mt. Kornuty, Beskid Niski Mts., W. Carpathians; **9** – Dolina Kościeliska Valley, Tatra Mts., W. Carpathians; **10** – Velký Kotel, Hruby Jeseník Mts., E. Sudetes.

population (Ojców) and the two W. Carpathian's populations form the Gorce and Pieniny Mts. The Cytotype III was found only in the Sudetes Mts. The Cytotype IV was the most diversified and weakly supported (37%), and found in the Slovakian part of the Carpathians in the Stražovské vrchy Mts., in central and eastern part of the W. Carpathians in the Tatras and Beskid Niski on Mt. Kornuty, and in the Silesian Upland (Fig. 2). Mt. Kornuty and the Tatra's population formed the best supported sister group with 73.7% bootstrap.

A PCR-ISSR analysis enabled the two main groups of localities to be distinguished. The first (group A) consists of the populations from the Małopolska Upland and the Polish W. Carpathians (Tatra, Pieniny, Gorce, Beskid Niski Mts.). The second (group B) was built of the two populations from Slovakia, namely from the Moravskoslezské Beskids and Stražovské vrchy Mts. (Fig. 2).

Hybrid zone of *Aconitum lasiocarpum* and *A. variegatum*

On northern slopes of the Polish Carpathians the two distinct species of *Aconitum* finish geographical ranges: *A. lasiocarpum* (Rchb.) Gayer and *A. variegatum* (SUTKOWSKA *et al.*, in print). In the overlapped areas a taxonomic hybrid *A. xpawlowskii* occurs (*A. lasiocarpum* × *A. variegatum*, Fig. 3 a, b). We test a hypothesis on the existence of a hybrid zone in the overlapping areas of *A. variegatum* and *A. lasiocarpum* subsp. *kotulae* (Pawl.) Mitka & Starmühl. in the Western Carpathians with the use of PCR-ISSR fingerprinting. The band-based nonmetric multidimensional scaling ordination showed the pattern of genetic population differentiation (Fig. 4). The distant populations of *A. variegatum* from Dąbrowa Górnicza (D – Silesian Upland) and Mt. Kornuty (K – Beskid Niski Mts.) are very close each to other. Similarly, all specimens of *A. lasiocarpum* are genetically very similar, however in their group three OTUs

Fig. 3. Geographic localization of the 6 sampled populations of *Aconitum* sp. in southern Poland (SUTKOWSKA *et al.*, in print).

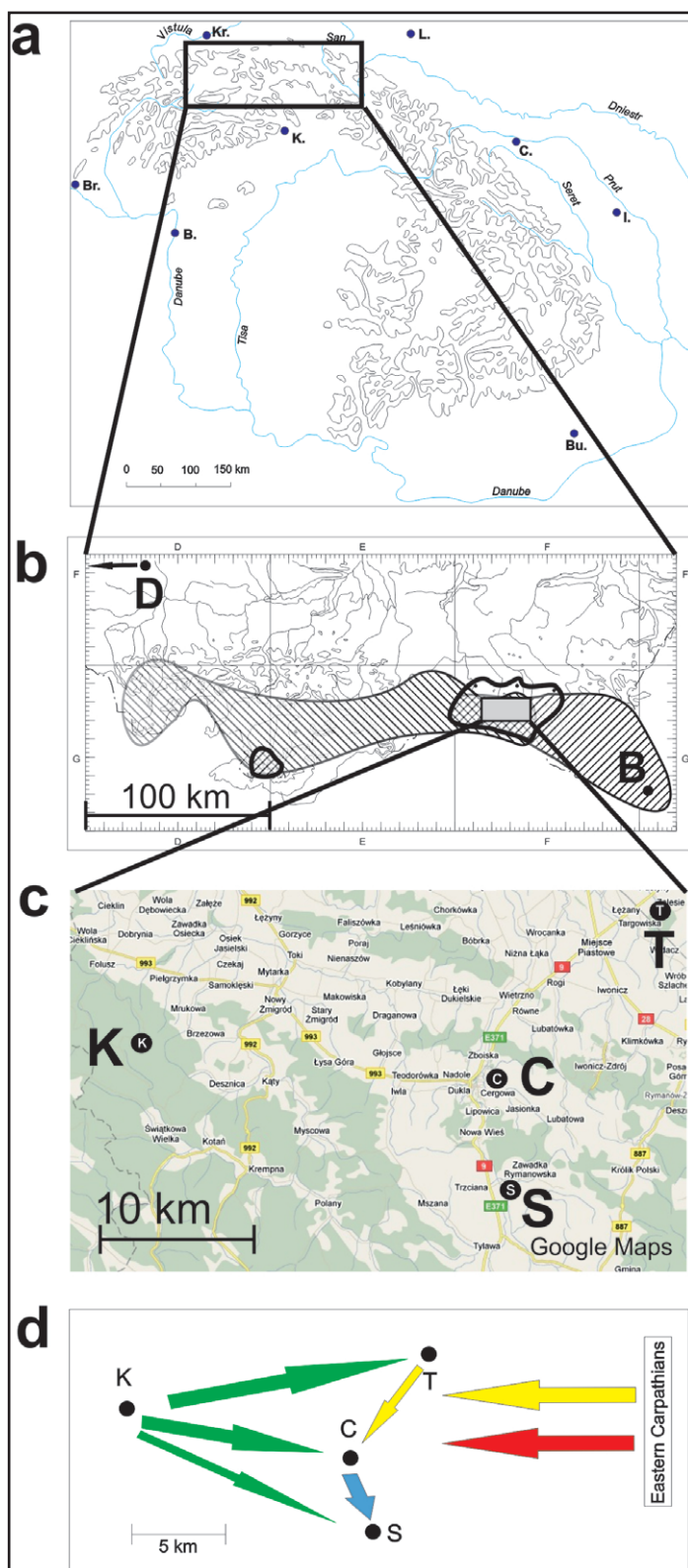
a. The Carpathians between Vistula and Dniester rivers, **B.** – Budapest, **Br.** – Bratislava, **Bu.** – București, **C.** – Chernivtsi, **I.** – Iassy, **K.** – Košice, **Kr.** – Kraków, **L.** – Lviv.

b. Distribution of *A. variegatum* (▨) and *A. lasiocarpum* (▧) populations, with the sympatric areas of *A. xpawlowskii* (○) occurrence, **D** – Dąbrowa Górnicza (Silesian Upland), **B** – Western Bieszczady, **E.** Carpathians (Sianki locality).

c. **C** – Mt. Cergowa (W. Carpathians), **K** – Mt. Kornuty (W. Carpathians), **S** – Stasiana (W. Carpathians), **T** – Targowiska (W. Carpathians).

d. Inferred migrations of *Aconitum* from STRUCTURE (PRITCHARD *et al.* 2000) results.

Taxonomic nomenclature after MITKA (2003). Distribution maps courtesy of ATPOL (ZAJĄC & ZAJĄC 2001).



of *A. xpawlowskii* are found. Two specimens from Mt. Cergowa (C) and Targowiska (T) located in the middle of the diagram, however taxonomically different, were also genetically similar. Seven specimens, marked with the star, were recognized by NEWHYBRIDS analysis (ANDERSON & THOMPSON 2002) as B1 introgressants (*A. lasiocarpum* backcross, i.e. $F_1 \times A. lasiocarpum$): five specimens of *A. variegatum* and two specimens of *A. xpawlowskii* (Fig. 4). The results support the view on the existence of natural interspecific hybrid swarm zone in the overlapped areas of *Aconitum* occurrence and gave argument for the taxonomical circumscription of the nothospecies.

Discussion

All of the presented analyses point to clear genetic and cytogenetic differentiation of *Aconitum* species along the W. Carpathians and Małopolska Upland. On the other hand, the genetic similarities may aid inference on their presumed Holocene migration routes from distant or nearby glacial forest refugia. Both *A. moldavicum* and *A. variegatum* can be regarded as forest species, with wide ecological profiles that enable them to survive the harsh climatic conditions. The regional population-(cyto)genetic pattern of *A. variegatum* in W. Carpathians and adjacent the Silesian and Małopolska Uplands can be an effect of at least two short-distance dispersal events, setting up (excluding the Sudetes) the geographic-genetic groups of localities. The first is presumably linked with the Holocene migration of the species (Cytotype I, see Fig. 2) along the Moravian Gate from the Moravian refugium. MAGRI *et al.* (2006) postulated here the existence of full-glacial refugium for *Fagus sylvatica* L. The second joined the Małopolska's population from Ojców with the W. Carpathian regions – Pieniny and Gorce Mts. (Cytotype II). This result posed a question on the status of the *Aconitum* populations in the Małopolska Upland, refugial or migratory. The results of the other studies on *A. moldavicum* are partially concordant with the refugial hypothesis. Also, the history of the vegetation reconstructed

with aid of the isopollen method (RALSKA-JASIEWICZOWA *et al.* 2004) seems relevant in this context. A tree species, hornbeam *Carpinus betulus* L., ecologically similar to *A. moldavicum*, recolonized the Małopolska Upland some 4000 years BP, according to the palaeobotanical evidences. Thus, presumed tempo of the tree migration from the putative Eastern-Carpathian – Volhynio-Podolian refugium may be estimated on about 56 km/year, in a time-span of 7 thousand calibrated years and in a distance of ca. 400 km. In comparison to wind-dispersed tree, the barochoric *A. moldavicum* had to a considerably lower tempo of migration, presumably close to 35 km/yr, that gives in the same time-span a distance of ca. 250 km. It points to its migration from the Beskid Niski Mts., but not from Podolia (MITKA & KOZIOŁ 2009). If these crude assumptions are true, then we can infer the existence of the species cryptic refugia in some parts of the Małopolska Upland (Dolina Będkowska, see Fig. 1). The conclusion is supported by the fact that the region – lying north of the Carpathians – is seriously taken into consideration as a forest faunistic refugium. For example, in the Nietoperzowa and Mamutowa Caves in Ojców the remnants of forest animals, dated to the last pleniglacial, were found, for example: *Clethrionomys glareolus*, *Lyurus terix*, *Discus ruderalis*, alongside with such typical for glacial species as *Ursus spelaeus*, *Coelodonta antiquitatis*, *Rangifer tarandus*, *Myopus* spp., *Ochotona* sp. and *Equus caballus* (PAWŁOWSKI 1991).

The studies on the natural hybrid zone in the Beskid Niski and Doły Jasielsko-Sanockie Depression (W. Carpathians) indicate that the population in Targowiska (T) developed as a result of migrations from different refugial centres (Fig. 1 d). The population is genetically the most variable and consists presumably of two migratory elements: one from the west of *A. variegatum* and another from the east of *A. lasiocarpum*. The population seems rather of a recent, namely the Holocene age. The other high-elevation population of *A. variegatum* in Mt. Kornuty (K) is taxonomically and genetically uniform. It could be an effect of its geographical isolation, and, maybe, a result of its

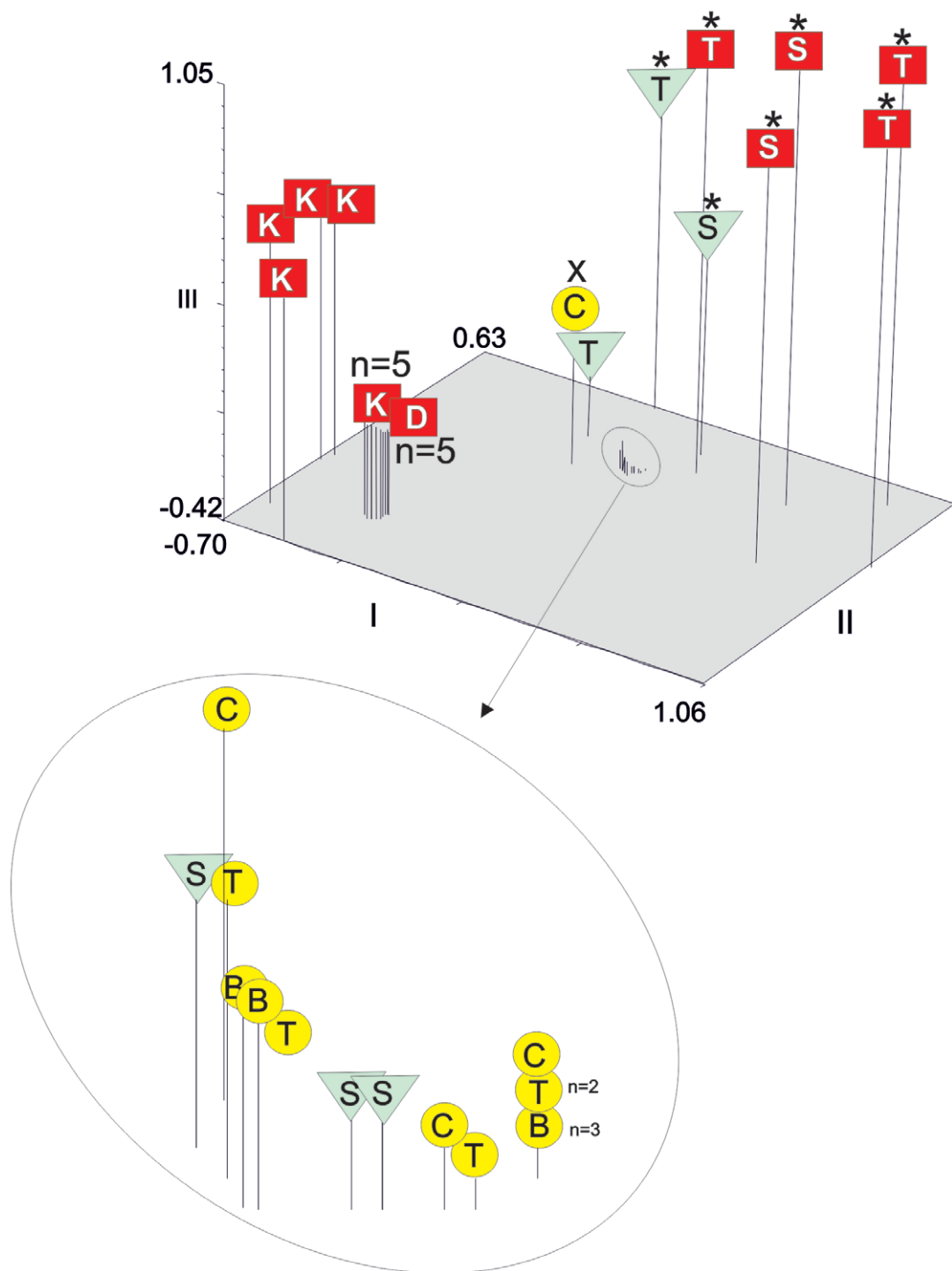


Fig. 4. Nonmetric multidimensional scaling according to NTSYSpc (ROHLF 2002), Stress = 0.100: ★ – genetic B1 hybrids according to NEWHYBRIDS analysis (ANDERSON & THOMPSON 2000); ✕ – introgressed *A. lasiocarpum*; □ – *A. variegatum*; ○ – *A. lasiocarpum*; ▼ – *A. xpałowskii*. For locality abbreviations see Fig. 3.

refugial character (Fig. 2). The lack of important cytogenetic difference between distant *A. variegatum* populations from the Beskid Niski (Kornuty) and Silesian Upland (Dąbrowa Górnicza, see Fig. 4) agreed with the existence of the widely distributed in Central Carpathians the Cytotype IV (Fig. 2). Maybe, it is dealt with its ancient, pre-glacial relict character recorded in the conservative cytogenetic marker.

The close relationships between the Małopolska Upland (Ojców) and W. Carpathians (the Pieniny Mts.), seen in the distribution of the Cytotype II of *A. variegatum*, turns our attention to the second presumably refugial region, i.e. the Pieniny Mts. (Fig. 2). Also, ISSR markers of *A. moldavicum* showed the genetic similarity between the Slovak and the Pieniny's population (Fig. 1). The results of the phylogeographical ISSR analysis of *A. moldavicum* points to limited influence of the Slovak populations on the origin of the island distribution of *A. moldavicum* in the Małopolska Upland and to probably refugial character of the Pieniny's population. On the other hand, the Małopolska's populations have another main source from the Beskid Niski Mts.

In the Polish W. Carpathians the Pieniny Mts. are a good candidate for a role of the cryptic, forest glacial refugium. This mountain range, however small and not too high (Mt. Wysokie Skalki, 1052 m a.s.l.), forms a distinct Carpathian geobotanical region and local center of endemism (PAWŁOWSKI 1972; ZARZYCKI 1976; TASENKEVICH 2005). The complicated relief of the mountain range and relatively long, 20 km distance from the Tatra Mts., give additional arguments. A very characteristic feature of the mountain range is its calcareous geological substratum and high rocky walls up to 150 m of relative altitude surrounding deep and picturesque the Dunajec River break. The complicated relief makes the area very rich in habitat types and hence the observed richness, relict and endemic character of the flora (ZARZYCKI 1976, 1981). In the Pieniny Mts. the Eemian forest refugium was previously postulated by ŚRODOŃ (1982). For example the last glacial maximum has persisted here *Juniperus sabina* L., the southern European

geographic element, noted in the Eemian flora of Gánovce near Poprad in Slovakia (KNEBLOVÁ 1960). Because the Pieniny Mts. are generally deprived of palinological deposits, the vegetation history here can be inferred only with a great caution. However, it is known that in some Carpathian valleys of Poland the last glaciation have survived several tree species of temperate-boreal type: *Larix decidua* Mill., *Picea abies* (L.) H.Karst., *Pinus cembra* L., *P. sylvestris* L. and *Populus tremula* L. (RALSKA-JASIEWICZOWA *et al.* 2004).

Conclusion

The existence of the cryptic forest glacial refugia being the shelter for forest vegetation in S. Poland, including the Małopolska Upland and the W. Carpathians, is highly probable. Generally, the problem has not been inquired to date, excluding the presented here preliminary results of studies on the genus *Aconitum*. The putative forest cryptic refugium in the Małopolska Upland could be presumably linked with another refugium in the Pieniny Mts. They might be of the same age since they represent genetically similar floristic elements, being the remnants of the interglacial Eemian forest flora of S. Poland. This hypothesis should be checked based on the representative samples of various forest species with different modes of reproduction and seed dispersal. The alternative hypothesis states that the one of the regions (preferably the Pieniny) was the glacial forest cryptic refugium, while the Małopolska Upland is the place of Holocene migrations from the Carpathian refugia. The other refugial area was Podolia-Volhynia region in Ukraine. *A. moldavicum* showed some links between the Podolian and Małopolska's regions, however the problem needs further studies based on a more representative plant material.

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